Table 1 Analyzed CD44, primer sequences and restriction enzyme.

Genes	Location of polymorphisms	Enzyme	Forward-primer	Reverse-primer
CD44 (rs7116432)	Exon 19 þ779A>G	NlaIII	CATCGTCTTCTTGCTGTTAGGA	GGTCTTGGTTCAGGTAGGGAGA
CD44 (rs187116)	Intron 1 þ4883G>A	MspI	AGGTGGTTGGAGATCACCTG	CTTTCGCAAGAACCACTTCC

Extended Abstract

Introduction: Approximately more than 930,000 cases were diagnosed with gastric cancer throughout the world. Overall Survival (OS) in patients with gastric cancer is less than 14 months. In Iran, North and North West regions are at high risk of gastric cancer. Significant differences in the frequency of environmental risk factors give the country an opportunity to investigate the causes of stomach cance. CD44 is the main molecule for cellular connections and it plays an important role in a number of physiological processes including cell attachment, migration and growth regulation. In humans, the CD44 antigen is encoded by the *CD44* gene on Chromosome 11. Moreover, CD44 increases proliferating tumor cells in cooperation with membrane-bound receptor tyrosine kinases containing C-src and C-erbB-2 (HER2/neu). Another important function of CD44 is to regulate tumor cell binding and motility. CD44 is involved in cell growth and metastasis formation of gastric and colorectal cancer. According to the study in California of single nucleotide polymorphisms rs187116 and rs7116432 with TTR and OS, we investigated the presence of these polymorphisms among patients with gastric cancer in Iran. We also investigated whether there is a strong significant association between these polymorphisms and clinical and pathological features of patients.

Material & method: 150 patients with gastric cancer and 150 healthy subjects participated in the study. All patients in Imam Reza and Omid Hospitals of Mashhad University of Medical Sciences, from 1998 to 2010, were treated through surgery only or surgery with (radio) chemotherapy. The study was approved by the Ethical Committee of Mashhad University of Medical Sciences (MUMS) and consent forms from all patients were obtained before any procedures. Patients' formalin-fixed paraffin-embedded (FFPE) tissues and healthy people's blood samples were taken. Then genomic DNA was extracted using an *extraction kit (QIAamp)* according to the manufacturer's protocol. Samples were tested by restriction fragment length polymorphism (PCR-RFLP) analysis or direct sequencing. Genes, *SNP reference identification numbers*, location SNP, SNP performance, forward primer, reverse primer and restriction enzymes are summarized in Table 1.

Joint and functional polymorphisms were selected in the *CD44 gene* according to previous articles. Our criteria for selecting a candidate gene polymorphism are as follows: low frequency of alleles 10% $\leq$ (MAF) in Caucasians and significant relationship with tumor reccurence and overall survival (Table 1). The primary endpoints of the analysis of polymorphisms in CD44 gene, included the time to tumor *recurrence (TTR)* and overall survival (OS), calculated from the date of diagnosis to date of the first observation of tumor recurrence or last follow-up, and the last case was used when the patient did not undergo tumor recurrence at that time. The TTR was calculated from the date of diagnosis of the disease to the date of first observation of tumor recurrence or until last follow-up if the patient was recurrence-free at that time. The OS wad defined as the time between disease diagnosis and death time to any cause or if the patient was alive until the last call. Distribution of polymorphism in connection with the main clinical and pathological demographic characteristics using the chi-square test was investigated. *OS* and *TTR curves* were analyzed based on COX regression. All statistical tests are two-way and done using the *SPSS Version 18.* Mann-Whitney test, which is a non-parametric test, was used to measure the age difference between patients and healthy subjects. The Kruskal-Wallis test, which compares three or more independent groups, was used for age differences and different states of single nucleotide polymorphisms. Finally, to determine the factors that affect the survival, Log Rank test were used.

Result: This research confirms the results by Winder et al in California that showed a strong significant association between rs187116 and rs711643 with TTR and OS in Iranian population. The presence of at least one allele G for Polymorphism rs187116 was significantly associated to decreased TTR and OS (P<0.0001). The presence of at least one allele A for Polymorphism rs7116432 was significantly associated to decreased TTR and OS (P<0.0001). We can use these results for prognosis and early detection of patients at high risk for tumor recurrence as an independent variable. Patients with diffuse type has a two-fold increase in recurrence rate compared to patients with intestinal yype (P<0.0001). However, type of tumor has no effect on OS.

Discussion: CD44 is a very complex molecule with different roles in cancer proliferation, invasion and metastasis. Recently collected evidence suggests that this molecule is a *gastric cancer stem* cell *marker.* It has been proven that overexpression of *CD44 variant* exons *V8*, *V9* and *V10* is associated with prognosis of gastric cancer. Previous studies have shown that CD44 gene polymorphisms can affect the survival of cancers. The results of this study demonstrate that there is a significant relationship between single nucleotide polymorphisms rs7116432 and rs187116 and gastric cancer. These results show that the patients' clinical outcomes can be predicted with CD44 gene polymorphisms. The new findings prove that intron changes may impact on the splicing regulatory elements that cause the aberrant splicing. Patients with G/G genotype of CD44 rs187116 had shorter OS than patients with genotype A/A. The findings may help select patients who are at high risk for recurrence so that thay can benefit from specific therapeutic strategies.

Keywords: Gastric cancer, CD44gene, Single nucleotide polymorphism, RFLP-PCR

Targeting colorectal cancer stem cells with nanoparticle

Armin Imanparast [a], Leila Hamzehzadeh [b], Alireza Pasdar [a,b,c,*]
[a] *Department of Medical Physics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, IR, Iran* [b] *Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, IR, Iran* [c] *Division of Applied Medicine, Medical School, University of Aberdeen, Foresterhill, Aberdeen, UK*
E-mail address: pasdara@mums.ac.ir

Extended Abstract

Introduction: Cancer is a leading cause of death worldwide. Due to recent improvements in early detection and treatment, the mortality rate has decreased in recent years, but treatment of many cases remains quite sophisticated. Colorectal cancer is the third major cause of cancer deaths in the United States both in men and women. Novel therapeutic strategies are under investigation including using nanoparticles. In this brief review different aspects of using nanoparticles in colorectal cancer stem cell are discussed.

Cancer stem cell: Cancer stem cells (CSCs) are small subpopulations of tumor cells responsible for the spread of cancer and tumor growth in a very efficient manner. Perhaps one of the most important causes of relapse and resistance to treatment is that the CSCs are not affected by common therapeutic agents. CSCs have the ability of infinite self-renewal and the capacity to differentiate into the various populations of cells that comprise a tumor. Self-renewal refers to the ability to create new stem cells with the same potential for proliferation, development, and differentiation, thereby maintaining the stem cell population. Induction of cancer stem cells to differentiate may result in removal of their ability of self-renewal. A niche is a special microenvironment where stem cells are stored and characterized by having different factors that can control stem cell proliferation and determination. In general, the niche is kept in a state of stasis by creating a signal that inhibits the growth and proliferation of stem cells. Stem cells can only get activated in presence of the excitation signals which make them start dividing and going into proliferation. So the balance between proliferation and inhibitory signals is the key for stem cell homeostasis. Any disruption increases the risk of developing tumors.

Colorectal cancer stem cells and their markers: Several studies identified a subpopulation of colorectal cancer cells that are more resistant to cancer treatments (such as chemotherapy and radiation therapy). Effective treatment depends on the elimination of these resistant subpopulations. These cancer stem cells or tumor-initiating cells have several highly expressed markers on their cell surface. Important markers in colorectal cancer stem cells are CD24, CD29, Lgr5, CD133 and CD44.

CD24 is a cell surface protein that is attached to the outer plasma membrane. CD24+ subpopulations have cancer stem cell-like properties such as increased resistance to chemotherapy, self-renewal and tumorigenic potential both in vitro and in vivo, compared to CD24- cell subpopulations.

CD29 (B1- integrin) is a member of the integrin family and contains a large extracellular and a short cytoplasmic domain that acts as a receptor for extracellular matrix proteins. It also activates signaling pathways to regulate cell migration, proliferation, permanence, differentiation and death. CD29 also plays a role in colorectal cancer cell metastasis.

LGR5 is a member of the Wnt signaling pathway. Although its ligand is unclear, it is one of the stem cell markers in the intestinal crypts. The findings show that LGR5 could play a key role in the development of CRC and may be considered as a useful marker for identifying and / or targeting colorectal cancer stem cells.

CD133 (or prominin-1) is a glycoprotein which is encoded by the *PROM1* gene in humans. While the accurate function of CD133 is not clear yet, it appears to act as an organizer of the cell membrane topology. However, the findings indicate that CD133 plays a key role in the initiation and progression of colorectal cancer and can be used as a marker of prognosis and diagnosis of CRC. CD44 is a cell adhesion molecule that plays an important role in facilitating cell-cell and cell-matrix interactions by connecting to its ligand such as hyaluronic acid. CD44 also facilitates the assembly of growth factors on the cell surface. Research has shown that CD44 could have a more decisive role in the tumorigenesis of colorectal cancer cells. Furthermore, active participation in many cellular activities, such as survival, differentiation, and migration are some other properties of CD44.

An effective treatment should be able to target all the different microenvironments of colon cancer stem cells in tumors to inhibit primary tumor growth, metastasis and recurrence of cancer. A promising method for increasing the efficiency of the treatment, is the use of nanoparticles in the diagnosis and treatment process. Biocompatible nanoparticles are conjugated with specific monoclonal antibodies against CCSC markers and also are capable of carrying drugs or small selectively directed RNAs to silence fundamental molecules for CCSC survival.

There are monoclonal antibodies such as specific anti-human CD133 mAb, anti human CD29 monoclonal antibody-clone 12G10, SWA11 mAb and KM4056 mAb for CD133, CD29, CD24 and Lgr5 antigens respectively. For a more effective and specific drug delivery, it is suggested to target colorectal cancer stem cells with specific antibody conjugated with nanoparticles which contain loaded drug.

Inhibition of the expression of *P53, HIF-1α and COX-2* genes as well as inhibition of NF-kβ, Wnt / β-catenin and K-Ras signaling and also activation of TGF-β are strategies for the treatment of colorectal cancer. Pending strategies for eradication of colorectal cancer stem cells are as follows:

1) PI3K signaling impairment causes Inhibition of proliferation and apoptosis induction

2) Hedgehog signaling impairment causes proliferation impairment and apoptosis induction

3) Notch signaling impairment causes a decrease in tumor growth and reduction in CCSCs frequency

4) Inhibition of IL-4 signaling causes sensitization to chemotherapy

5) CD44 silencing causes apoptosis induction and tumor growth suppression [19].

Nanotechnology and regulation of gene expression: NanoScript is an artificial nanoparticle with similar functional Transcription Factor (TF) protein that in fact is a platform for regulating gene expression. TF is a binding protein to specific sequence of DNA, so regulates the rate of transcription of genetic information from DNA to messenger RNA. NanoScript has two interesting features that make it appropriate platform in the field of stem cells: First, it is non-viral, that is a good alternative to viral vectors. Second, simple arrangement of the sequence of any molecule on NanoScript is possible so they can specifically target and induce differentiation.

Conclusion: In this review, we discussed the important role of cancer stem cells in cancer recurrence, Then examined the role of genes and signaling pathways in the progression of colorectal cancer and it was shown that with the use of nanotechnology can be affected the expression of genes and signaling pathways in cancer therapy to specifically target cancer stem cells and lead to increased efficiency of treatment.

Keywords: Colorectal cancer, Cancer stem cell, Cell signaling, Nanoparticle, CD133, CD24, CD29, CD44, Lgr5